REMARKS

Applicant respectfully requests reconsideration. Claims 1-10, 20, 25-27, and 29 were previously pending in this application. By this amendment, Applicant is canceling claims 6-10, 20, 25-27, and 29 without prejudice or disclaimer. Claims 1 and 2 have been amended to indicate that the mood disorder is a bipolar disorder. As a result, claims 1-5 are pending for examination with claims 1 and 2 being independent claims. No new matter has been added.

Claim Objections

The Examiner objected to claim 20 being a claim to a product that improperly depended from a claim to a method in which that product might be used. Applicants have cancelled claim 20, which obviates the rejection.

Rejections under 35 U.S.C. §112

The Examiner rejected claims 1-10, 20, 25-27 and 29 under 35 U.S.C. §112, first paragraph as failing to comply with the enablement requirement.

Applicants have amended claims 1 and 2 and believe claims 1-5 are fully enabled by the application as filed. Claims 6-10, 20, 25-27 and 29 have been cancelled.

Applicants respectfully submit that the specification as filed contained sufficient technical teaching to enable one skilled in the art to identify coding regions within the region of chromosome 18 delineated by markers D18S68 and D18S979 through the application of routine procedures, without undue burden. In the present application Applicants have provided the identification of a specific region of human chromosome 18q that shows linkage with bipolar disorder. Based on this information provided by Applicants, it is a matter of routine experimentation for one of skill in the art to identify the specific polymorphisms and genes within this region that are *associated with* bipolar disorder.

Claims 1 and 2 as amended relate to methods for identifying coding regions/genes that are associated with bipolar disorder. There is no reference in these claims to achieving *diagnosis* of bipolar disorder; the claims merely relate to identification of genes that are associated with the disease. Applicants submit that the specification as filed describes various techniques, all well

known in the art at the priority date, that one of skill in the art could apply in order to utilize the information Applicants have provided in the form of the identification of the linkage region on human chromosome 18q, to identify genes and polymorphisms associated with susceptibility to bipolar disorder. Furthermore, with the publication of the human genome sequence, *in silico* characterization of the specific chromosomal region identified by the inventors in the specification as filed is now facilitated.

Applicants submit that any suitable technique known in the art for identifying candidate coding regions/genes could be used in order to identify coding regions/genes in the linkage region identified by Applicants in the instant specification. A number of particularly suitable techniques are described in further detail in the passages from page 14, line 19 to page 22, line 4. Methods for identifying candidate bipolar disorder genes using the preferred techniques recited in these passages all share the following procedural steps:

i) Identify positions of coding regions/genes in the DNA that can be compared to the equivalent regions of DNA from a person afflicted with bipolar disorder;

ii) Detect differences between said coding regions/genes and equivalent regions in the DNA of an individual afflicted with bipolar disorder; and

iii) Thereby identify a gene (or mutated or polymorphic variant thereof) that is associated with bipolar disorder.

Any suitable technique may be used in part i) in order to identify positions of coding regions/genes in the DNA. However, particularly preferred techniques described in the present application include exon trapping (see page 16, lines 8 to 34), subcloning into a vector followed by construction of a contig map and identification of the positions of possible genes using techniques such as cDNA selection or capture, hybridization to mRNA/cDNA, CpG island identification, or zoo-blotting (see page 17, line 31 to page 20, line 1), or computer analysis (see page 20, lines 3 to 6).

In order to carry out part ii) there are many known techniques that can be used to make detailed comparisons between candidate genes from a normal individual and an individual afflicted with bipolar disorder. Suitable techniques include, for example, mutation testing, southern blotting, heteroduplex mobility in polyacrylamide gels, single-strand confirmation or polymorphism analysis, chemical cleavage of mismatches, enzymatic cleavage of mismatches, denaturing gradient gel electrophoresis or direct DNA sequencing (see page 20, line 7 to page 22, line 3).

Because genetic linkage between the relevant region of human chromosome 18 and bipolar disorder has been shown by Applicants to be significant, it is submitted that there would be a high expectation of success that an analysis of this region of chromosome would lead to the identification of polymorphisms and genes that are associated with susceptibility to disease. Now that the very specific region of the genome has been shown by Applicants to be associated with bipolar disorder, one of skill in the art could use that information to search for, and indeed would expect to find, polymorphic alleles and genes that are associated with susceptibility to bipolar disorder. The amount of experimentation may be significant, depending on the method chosen. However, such experimentation is routine in the art and at no point would represent any undue burden for the skilled person. Applicants submit that the level of skill in the art of genetic manipulations and screening is quite high with most practitioners holding either an MD or a PhD in fields such as molecular biology and/or genetics. The methods described in the specification for identifying coding regions/genes associated with bipolar disorder, now that Applicants have provided the starting region, are well within the normal practice and routine for those of ordinary skill in the art in these fields. Thus, Applicants submit that the specification as filed provides ample guidance for one of ordinary skill in the art to identify genes associated with susceptibility to bipolar disorder, now that Applicants have identified the region of chromosome 18 to be examined.

Once one of skill in the art has identified coding regions and/or polymorphisms in the defined region of chromosome 18 it is also a routine matter for one of skill to identify whether a particular coding region is *associated with* bipolar disorder.

The Examiner asserts at page 7 of the Office Action that "the art is unpredictable with respect to identifying genes associated with disease and detecting the presence of novel mutations associated with the occurrence of disease is highly unpredictable". Assuming arguendo that the publications cited by the Examiner in support of this statement generally indicate that it is difficult to identify genes associated with disease, Applicants submit that the publications do not support a conclusion that the effort and experimentation required would be undue or would be beyond the scope of work undertaken routinely by those of ordinary skill in these arts. To practice the claimed invention, one of ordinary skill need only utilize standard molecular biology procedures, in conjunction with the information provided by Applicants that identifies the region of chromosome 18q.

Applicants submit that this assertion is supported by the Gerson publication cited by the Examiner in that Gerson indicates that chromosome 18 is "one of the best candidate locations for a bipolar susceptibility gene" and "represents important progress". The instant invention is based in part on Applicants' identification of a more specific and defined candidate region than previously available and thus advances the art toward identifying bipolar associated genes by providing methods with which one of skill in the art can use the new regional information to identify more specific regions and candidate genes associated with bipolar disorder.

The Examiner also cites a publication by Lucentini as indicating the unpredictability in the art. Applicants submit that the statement in the Lucentini publication that "there may be no way to predict which new gene-associate studies will be verified with multiple replication" does not mean that one of ordinary skill in the art would not expect to be able to utilize the information on the candidate region of chromosome 18 provided in the instant application to identify coding regions and/or genes that are associated with bipolar disorder as claimed. As also stated in the Lucentini publication, "researchers should treat any finding cautiously until it's replicated, preferably more than once", thus restating a standard practice of those of ordinary skill in the art to base conclusions on repeated testing. Applicants submit that this discussion in Lucentini does not imply that one should not expect to find coding regions and/or genes associated with bipolar disorder as set forth in the claimed methods, but rather one should interpret their results carefully. Applicants submit that this does not reflect on the predictability of the art as much as it simply indicates that care must be taken to ensure confidence in an

association identified between a mutation/polymorphism and the occurrence of a disease or condition.

With respect to the significance of linkage in family MAD31, Applicants submit that one of ordinary skill in the art would recognize the results set forth by Applicants in the application as evidence of linkage. Applicants submit that LOD scores and p-values are two different ways of expressing the results of a linkage analysis statistically and that one of ordinary skill in the art would realize that a LOD score of 1.2 is equivalent to a p-value of 0.01. It is not necessary for both values to be given in order for a skilled reader to assess the significance of a particular linkage study. A LOD score above the threshold of 1.2, equivalent to a p-value of ≤ 0.01, would be accepted as evidence of significant linkage by one skilled in the art in the type of study carried out by Applicants. As discussed in the response filed January 18, 2005 Applicants did not carry out a full genome scan, but rather a multipoint linkage analysis using STR markers from a particular region of chromosome 18. Therefore the threshold for significant linkage is not a LOD score of 3.0 as would be appropriate for a genome-wide scan, but is set at a lower value. Applicants assert that one of ordinary skill in the art would recognize this difference, which is based on the fact that a smaller region of the genome is being scanned, hence the probability of a false-positive p-value occurring within the region by chance is lower than it would be for a whole genome scan. In further support of the significance of Applicants' linkage analysis and the LOD values presented in the specification as filed, Applicants provide herewith a Declaration by Professor Christine Van Broeckhoven regarding the statistical significance of Applicants' analysis and their identification of the region of human chromosome 18q disposed between polymorphic markers D18S68 and D18S979 as associated with bipolar disorder.

Applicants note that the issue raised by the Examiner regarding the predictability in the use of linkage results obtained with family MAD31 as applicable in other mood disorders or to other related disorders, has been obviated by the amendment of claims 1 and 2 to recite bipolar disorders rather than the more extensive listing of disorders. Accordingly, the issues raised by the Examiner in relation to the report by Nancarrow regarding association between bipolar susceptibility regions on 18q or 18p and susceptibility to schizophrenia are no longer applicable to the pending claims.

The Examiner notes the Lander reference statement that "replication studies should always state their power to detect the proposed effect with the given sample size" as indicative that the significance of the disclosed LOD scores cannot be properly evaluated. (Office Action at page 15-16). Applicants submit that this statement by Lander is simply a personal view from the authors of the Lander article and does not affect the significance of the results presented in this application. Lander *et al.* themselves acknowledge that this does not always occur, (see page 245, column 1), hence it should not be viewed as standard practice in the art.

Applicants agree that it is correct that the size of the family and the number of families studied effects interpretation of linkage data. However, the fact that Applicants' study was carried out in a single Belgian family does not mean that the linkage result is not significant. The initial genome-wide linkage studies performed by the present inventors and others, which led to the finding of linkage with regions of chromosome 18, were carried out in a large number of families using a "non-parametric" approach, in which no assumptions are made regarding the mode of inheritance of the disease. It was in fact key to Applicants' success in narrowing down the region of linkage on chromosome 18 that they switched to linkage analysis based on a parametric approach in a single family (MAD31) which exhibits a near-Mendelian mode of inheritance for bipolar disease. Hence, even though the study was performed in a single family, it does not reduce the significance of the results and Applicants submit that one of ordinary skill in the art would not question the results of Applicants' linkage analysis purely because the study was carried out in a single family.

Applicants submit that the teaching of Goossens *et al.* is not relevant to a consideration of whether the present application provides sufficient technical teaching to enable one skilled in the art to identify a candidate gene for bipolar disorder. Goossens *et al.* did not follow the teaching of the instant application with the aim of identifying candidate susceptibility genes in the claimed region of chromosome 18. Rather, the aim of Goossens *et al.* was merely to investigate whether particular trinucleotide repeats in the claimed region on chromosome 18 show an association with bipolar disorder. Goossens *et al.* conclude that the particular CAG/CTG repeats analyzed in their study are unlikely to cause bipolar disease in the particular patients evaluated in that study. The results presented by Goossens *et al.* do not exclude the possibility that other susceptibility loci for bipolar disorder occur elsewhere in this region of chromosome 18, or even that the same

trinucleotide repeats are associated with or causative for bipolar disorder in other patients. Goossens *et al.* does not have any bearing on whether it is feasible to identify candidate genes in the newly identified region as claimed using routine techniques, such as those identified in the present application.

Applicants submit that the teaching of the McInnes publication is also not relevant to the issue of enablement. It is not necessary to carry out any further linkage studies in order to practice the methods of amended claims 1 and 2 because Applicants have already identified the linkage regions between chromosomal markers D18S68 and D18S979 and between chromosomal markers D18S60 and D18S61 as being regions to search for genes associated with bipolar disorder using the method of the invention. Applicants have already delineated a clear candidate region on chromosome 18.

With regard to the publication by Nothen *et al.*, Applicants do not consider that this citation is relevant to the issue of enablement of the instant claims. Nothen *et al.* is concerned with linkage studies aimed at re-examining linkage of bipolar disorder to markers on chromosome 18 in German families. The results of this study were found to be consistent with the existence of a bipolar susceptibility locus at 18q22-23. In fact, Applicants have already identified a clear candidate region on chromosome 18 from their own linkage studies in the family MAD31. Claims 1 and 2, as amended, relate to methods of identifying genes/coding regions associated with bipolar disorder within this region. In order to practice these methods it is not necessary to carry out any further linkage studies either to confirm linkage to the claimed region of chromosome 18 between markers D18S68 and D18S979 or to further narrow the candidate linkage region. Rather, as discussed below, based on the information provided in the specification regarding the region of chromosome 18q as associated with bipolar disorder, one of ordinary skill in the art can proceed straight to screening for coding regions within the region delineated by markers D18S68 and D18S979 with a reasonable expectation of success in finding a coding region associated with bipolar disorder.

Nothen *et al.* do not dispute that a bipolar susceptibility locus exists on chromosome 18, they merely conclude that it may be difficult to precisely locate the locus through further *linkage* studies. In fact, Applicants through their own linkage studies have narrowed down the candidate region on chromosome 18 sufficiently that it is not necessary to perform any further linkage

studies in order to further refine the candidate region. As discussed above herein, one of ordinary skill in the art can proceed straight to screening for candidate coding regions using the new regional information provided and the techniques listed in the specification as filed, which do not involve further linkage analysis. Applicants narrowed the candidate region on chromosome 18 to a region sufficiently small that they were able to provide a contig map of publicly available YAC clones containing the actual DNA of the region between markers D18S60 and D18S61, including the candidate region between markers D18S68 and D18S979. The availability of this contig map and the DNA present in the YAC clones further facilitates the identification of candidate coding regions using the claimed methods.

Applicants would also like to draw the Examiner's attention to published International patent applications WO 02/101044 (priority date June 11, 2002) and WO 03/025222 (priority date of September 17, 2001) as being directly relevant to sufficiency of the instant application with respect to the identification of candidate bipolar genes. Applicants provide copies of each published application herewith.

Referring first to WO 02/101044, the inventors set out with the intention of isolating candidate bipolar disorder genes from the region of chromosome 18 located between markers D18S68 and D18S979. In order to identify candidate genes the inventors utilized the experimental procedures outlined in the instant application. In particular, the inventors used CCG/CGG YAC fragmentation to construct sets of fragmented YAC clones and thus identified three potential CpG islands in the candidate region. One CpG island was found to be located upstream of a 3639bp exon using an exon-prediction computer program, which is an approach discussed on page 20, lines 3-6 of the present application. This exon was found to form part of a potential candidate gene denoted NCAG1.

Having isolated a potential candidate gene in the region the inventors set about trying to establish whether NCAG1 is associated with bipolar disorder. In order to establish an association the inventors of WO 02/101044 used mutation analysis of the coding region of NCAG1, which is precisely the technique discussed on page 20, lines 7 to 21.

Mutation analysis of coding regions is an accepted and legitimate approach to the identification of candidate genes associated with a complex genetic disease. This approach is based on the hypothesis that significantly less genetic variation is expected to occur within the

coding regions of a disease-associated gene, yet variants that do occur in the coding region might be expected to affect the function of the encoded protein. Hence, analysis of the coding regions is expected to identify less genetic variants, but it is more likely that one of the variants will show a significant genetic association with disease phenotype if the gene is indeed a candidate disease gene. It should be noted that mutation analysis is used for the purposes of proving a genetic association and it is not necessary to identify a genetic variant which is *causative* for disease at this stage.

Using the mutation analysis approach the authors of WO 02/101044 identified two single nucleotide polymorphisms in the coding region of NCAG1. In order to investigate whether either polymorphism in NCAG1 was significantly associated with bipolar disorder the authors carried out a population-based allelic association study in 92 bipolar patients (not related) and 92 age, sex, and ethnicity matched controls. The association study is merely a means of comparing a "difference" (i.e. a polymorphism) in a large number of affected and normal individuals and is an approach that was in routine use before the priority date of the instant application. No alleles, genotypes or haplotypes were found to be significantly associated with bipolar disorder, indicating that NCAG1 itself is not a candidate bipolar gene. Nevertheless, this result does not affect the validity of the experimental approach. A "true" candidate gene would be identified purely on the basis of a statistically significant association in the population-based association study. The experimental approach would be the same - only the result would be different.

With respect to the WO 03/025222 publication, the authors of this document identified a potential candidate gene denoted CAP2 in the region located between markers D18S68 and D18S979 using analogous techniques to those taught and described in the instant application. In order to establish whether CAP2 is a true candidate gene the authors used exactly the same approach as outlined in the instant application and used in WO 02/101044; namely, they carried out a mutation analysis in order to identify polymorphisms in the coding region (and one intron) of CAP2 and then compared "differences" at the polymorphic loci by genotyping a large number of affected and control individuals in a population based allelic association study in 75 unrelated bipolar patients and 75 matched controls. A slight departure from the Hardy-Weinberg equilibrium was observed for one polymorphism. More specifically the T allele of SNP c.942C>T had a significantly higher frequency in bipolar disorders.

The results of this study, in combination with WO 02/101044, illustrate that identification of a candidate bipolar gene is purely a matter of showing a statistically significant result when the presence of a "difference" (a polymorphism) is assessed in a large number of individuals, i.e. in an association study. The basic experimental approach was the same each time, namely: i) identify polymorphisms in the coding region of the potential candidate gene and ii) evaluate the polymorphisms in a population based association study. Thus, it is entirely possible for one skilled in the art to follow the teaching of the present application in order to isolate potential candidate bipolar genes in the claimed region of chromosome 18 and to test their association with bipolar disorder. The experimental approach required is exactly that set out in the instant application and is clearly not a matter of "random experimentation".

The applicant would also like to make available to the Examiner a review article by Cox and Bell, Diabetes, Vol.38, 1989. (copy provided herewith). This review article sets out the general approach that is taken in order to identify whether a particular "candidate gene" shows an association with a complex genetic disease, and hence is a true "susceptibility gene". Although this review is primarily concerned with diabetes the technical teaching applies also to other complex genetic diseases. In the section headed "Association study design and rationale" the authors state that association studies compare patient and control groups with respect to some marker. In order to investigate whether a particular coding region is a "susceptibility gene" an investigator need only assemble appropriately matched groups of patients and control subjects, isolate DNA, type the individuals and determine if the marker frequencies differ between the two groups. This is exactly the approach that was taken by the authors of WO 02/101044 and WO 03/025222 in relation to candidate bipolar genes. Cox and Bell confirm that this approach was general knowledge as early as 1989. The difference between success and failure in identifying a particular coding region as a "susceptibility gene" associated with a particular disease is purely a matter of statistics in the association study. Applicants submit that would not be considered to be "random experimentation".

It is to be noted that the inventors have not only identified the linkage region between markers D18S68 and D18S979 on chromosome 18, they have also constructed a YAC contig map of the linkage region and identified publicly available YAC clones as incorporating the candidate region. Thus, the inventors have provided the skilled reader with access to YAC DNA

clones that can be used to identify candidate genes, following the procedures outlined in the instant application and illustrated in WO 02/101044 and WO 03/025222.

Of course, having identified a gene showing a genetic association with bipolar disorder it is most likely that one of skill in the art would like to go further to establish the function of the gene and investigate how it may contribute to the bipolar phenotype using different experimental techniques. The experimental approach taken may vary considerably depending on the nature of the candidate gene, its expression patterns, homology to other genes of known function etc. Nevertheless, it should be noted that the present claims (as amended) relate to methods for identifying at least one human gene that is associated with bipolar disorder. Applicants would like to emphasize that claims 1 and 2 are not directed to specific genes or coding regions *per se* but rather relate to *methods* of identifying such genes or coding regions in the regions of chromosome 18 disposed between polymorphic markers D18S68 and D18S979 and between markers D18S60 and D18S61. The claims do not require the skilled reader to establish the precise function of the candidate gene or to show whether or how it is causative for bipolar disorder. The teaching of the present application enables the skilled reader to identify potential candidate genes and show whether or not they are genetically associated with bipolar disorder. Applicants respectfully submit that on this basis, the standard for enablement has been met by the specification as filed.

Applicants submit that it is also well within the capabilities of one of skill in the art to identify polymorphic variants within the claimed region on chromosome 18q that show a genetic association with bipolar disorder. The application as filed teaches several techniques that can be used to compare the claimed genomic region from one individual with that from any other individual and to observe differences, i.e. to identify polymorphisms. In order to show an association between a particular genetic variant in the region bounded by markers D18S68 and D18S979 and bipolar disorder it is not necessary to have access to material from a specific family (e.g. MAD31) or a specific group of patients. Rather, allelic associations can be shown across a population in a population-based association study that involves genotyping of both afflicted individuals and control individuals, who need not be related to each other, for the particular genetic variant. Such population based (or case-controlled) allelic association studies

were well known and in routine use before the priority date of the instant application. Moreover, it would be clear to what one skilled in the art that the term "associated with" refers to a genetic association and that such associations can be shown in via genetic association studies, which are merely a tool for comparing "differences" between a large number of affected and control individuals.

By way of illustration, the present application identifies several polymorphic markers that may serve as markers for bipolar disease. In particular, the inventors have shown that affected individuals share alleles at STR markers D18S969, D18S1113, D18S876 and D18S477. None of these markers has been shown to be "functional" in the sense that they form part of a candidate gene for bipolar disorder or somehow contribute to disease phenotype, yet any one or any combination of these markers can be used to test for susceptibility to bipolar disorder across the population.

In conclusion, Applicants submit that the claims as amended do bear a reasonable correlation to the scope of enablement because the specification provides the novel identification of a region of chromosome 18q that is associated with bipolar disorder and describes how the novel identification can be used to identify coding regions/genes or mutated or polymorphic variants within the newly identified region that are associated with a bipolar disorder. This novel identification of the region is sufficient to allow one of ordinary skill in the art to practice the claimed methods throughout their scope. As set forth above, Applicants do not claim methods to identify coding regions or genes that are diagnostic for a bipolar disorder or other mood or related disorder, but simply claim methods with which one of ordinary skill can identify coding regions/genes or a mutated or polymorphic variant associated with bipolar disease.

Applicants submit that a full consideration of the amended claims in light of the factors set forth in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (CAFC 1988), supports the conclusion that no undue experimentation is required for enablement. Applicant therefore respectfully requests the Examiner to withdraw the rejection of claims 1-5 under 35 U.S.C. §112 first paragraph.

Rejections under 35 U.S.C. §112

The Examiner rejected claims 1-6 and 8 under 35 U.S.C. §112, second paragraph as being indefinite. Applicants respectfully traverse the rejection. Claims 6 and 8 have been cancelled.

The Examiner states at page 18 of the office action that the meaning of "equivalent" as used in the specification and claims is not a definition known in the art to define equivalent nucleic acid regions. Applicants disagree with the Examiner's interpretation of the term "equivalent" and respectfully submit that the Examiner's interpretation does not correspond to what a skilled reader would understand by this term in the context in which it is used in the instant application and claims.

Applicants submit that based on a general knowledge of molecular genetics a skilled reader would not interpret this term as meaning (as the Examiner indicated in a previous Office Actin) that sequences from a particular area of the genome in one individual should be compared with sequences in another individual (possibly from an entirely different position in the genome) which have some "unstated degree of similar sequence". Applicants submit that one of ordinary skill in the art would recognize the art-known meaning of this term as referring to an equivalent physical location on chromosome 18q or a region that occupies the same genetic locus. Applicants submit that the term "equivalent region" is used extensively in the art of molecular biology and that its meaning is clear to one of ordinary skill. In support of the general understanding of this term among those of ordinary skill in the art, Applicants provide herewith a Declaration by Professor Christine Van Broeckhoven indicating that, at the time of filing, the term "equivalent region" would be an art-recognized term when used in reference to sequences and genetic loci and that the meaning of the term would have been known to one of ordinary skill in the art.

Accordingly, withdrawal of the rejection of claims 1-5 under 35 U.S.C. §112, second paragraph as being indefinite is respectfully requested.

CONCLUSION

A Notice of Allowance is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,
Christine Van Broeckhoven et al., Applicants

By: _____

MaryDilys S. Anderson, Ph.D.
Reg. No. 52,560
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
Telephone: (617) 646-8000

Docket No.: B0192.70019US00
Date: October 18, 2005
x10/18/05x